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Inhibitory effect of 4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole on HCMV DNA replication and permissive infection

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Abstract

We found that Human Cytomegalovirus (HCMV) infection of human fibroblasts resulted in a dramatic increase in p38 mitogen-activated protein kinase (MAPK) phosphorylation. Recently, drug mediated inhibition of p38 has been demonstrated to exhibit anti-viral activity against HIV (Shapiro, L., Heidenreich, K.A., Meintzer, M.D. and Dinarello, C.A., 1998. Role of p38 mitogen-activated protein kinase in HIV type 1 production in vitro. Proc. Natl. Acad. Sci. USA. 95, 7422–7426). Therefore, we examined the effect of a specific p38 kinase inhibitor on HCMV infection. Inhibiting p38 activity in HCMV infected cells by treating cells with 4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole; (FHPI), a p38 inhibitor drug, prevented permissive HCMV infection as measured by plaque assay. In the presence of FHPI, HCMV immediate early gene expression was slightly lower at early times of infection, but there was no inhibition of expression of the early gene UL-84, an HCMV protein essential for viral replication. However, FHPI inhibited HCMV DNA replication and late gene expression. The inhibitory effect of FHPI was reversible, as demonstrated by the induction of HCMV replication upon withdrawal of FHPI. Our data describes FHPI as a novel anti-HCMV compound that inhibits synthesis/activation of cellular and/or viral factors required for initiation of HCMV DNA replication.

Keywords: FHPI; Antiviral compound; Human cytomegalovirus (HCMV); p38 MAPK

1. Introduction

Human Cytomegalovirus (HCMV) is a ubiquitous herpesvirus that is associated with many

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clinical manifestations. In immunocompromised individuals, these manifestations are often serious and can be fatal (Huang and Kowalik, 1993). Currently, ganciclovir (DHPG), a nucleoside analog which targets the viral DNA polymerase, is the leading anti-viral drug used to control HCMV infection (Mar et al., 1983). Unfortunately, this drug can have harmful side-effects, such as thrombocytopenia, granulocytopenia, and anemia (reviewed in Serody and van der Horst (1993)). In addition, HCMV strains resistant to DHPG and other anti-HCMV drugs have emerged (Stanat et al., 1991; Baldanti et al., 1995a,b, 1996, 1998 (and references within)). The side effects caused by ganciclovir and the emergence of resistant strains make it imperative to identify new drugs that can be used to limit HCMV infection. In this report, we describe FHPI as a novel compound that inhibits HCMV infection.

A permissive HCMV infection consists of a series of sequential steps, with each step being dependent on the completion of the previous one (Stinski et al., 1991a; Mocarski, 1993). The first step is viral attachment to the host cell, followed by viral entry and uncoating. Next, the HCMV immediate-early (IE) genes are expressed from the major IE promoter (MIEP). The transactivation function of the IE gene products are required for expression of the early (E) genes, which is necessary for viral DNA synthesis. After DNA synthesis, viral late (L) genes are expressed, then the viral genome is packaged and infectious viral particles are released. Identification of the stage at which the viral life cycle is disrupted is an important step in identification of the mechanism of inhibition of any anti-viral drug.

HCMV infection can result in activation of many cytokines, including IL-1β and TNF-α (Dudding et al., 1989). An important component of IL-1β and TNF-α signaling is activation of the p38 mitogen activated protein kinase (MAPK) pathway (Raingeaud et al., 1995; Read et al., 1997; Larsen et al., 1998). Activation of this pathway results in phosphorylation of the p38 MAP kinase on Thr-180 and Tyr-182 (Raingeaud et al., 1995). Once phosphorylated, the kinase function of p38 is activated. p38 can then phosphorylate a variety of transcription factors, including members of the ATF/CREB family, which results in increased expression of many different types of stress response genes, including cytokines, such as TNF-α, and adhesion molecules, such as E-selectin (Lee et al., 1994; Raingeaud et al., 1995; Tan et al., 1996; Iordanov et al., 1997; Read et al., 1997). To date, four functional isoforms of p38 have been identified: p38α, p38β2, p38γ, and p38δ (Han et al., 1994; Lee et al., 1994; Rouse et al., 1994; Cuenda et al., 1997; Wang et al., 1997; Enslen et al., 1998; Nemoto et al., 1998).

Recently, p38 activation has been shown to be important in HIV infection of peripheral blood mononuclear cells. HIV infected cells treated with IL-1β or TNF-α dramatically increased HIV production, as measured by production of the HIV antigen p24 (Shapiro et al., 1998). However, inhibition of p38 kinase activity using the p38 inhibitor compound SB203580 prevented this increase. Interestingly, treatment of cells with SB203580 inhibited p24 synthesis even in cells that were not treated with IL-1β or TNF-α, suggesting that p38 activation plays an important role in normal HIV viral infection (Shapiro et al., 1998). Due to the many different functions of p38, and the number of substrates p38 can activate, it may be difficult to elucidate the precise role of p38 in this viral infection.

Two different compounds that inhibit p38 kinase activity have been identified: 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4pyridyl)-1H-imidazole and 4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole, referred to as SB203580 and FHPI, respectively (Lee et al., 1994). Neither prevents p38 phosphorylation; rather, they prevent p38 kinase activity by competing for the ATP binding site of p38 (Young et al., 1997). These drugs, both of which are pyridinyl imidazole compounds, specifically inhibit the two major p38 isoforms, p38α and p38β2, without affecting the two less prevalent form of p38, p38γ, and p38δ, or any of the other MAP kinases, such as JNK ((Nemoto et al., 1998) and references within).

Initially, we observed that HCMV infection could induce phosphorylation of p38. The main purpose of this study was to examine the anti-

HCMV properties of the MAPK p38 inhibitor compound FHPI. In addition, we examined which steps in the viral life cycle were inhibited by FHPI. In this report, we describe inhibition of HCMV permissive infection of human fibroblasts by the drug FHPI. FHPI inhibits the HCMV viral life cycle after IE gene expression but before viral DNA replication, and this inhibition is reversible.

2. Materials and methods

2.1. Drugs

FHPI (SB202190) (Fig. 1) was purchased from Calbiochem (La Jolla, CA). Ganciclovir (1,3-dihydroxy-2-propoxymethyl)guanine (DHPG)) was purchased from Syntex, Inc. (Palo Alto, CA).

2.2. Cell culture, viral passage

Human embryonic lung (HEL) fibroblasts were propagated in minimal essential media (MEM) (Gibco, BRL) supplemented with 10% fetal bovine serum (FBS) plus antibiotics. Towne strain HCMV (passage 36–40) was propagated in HEL cells as previously described (Kowalik et al., 1993).

2.3. Titer reduction assay

HEL cells were grown to confluency in 24-well plates and 24 h later, the cells were pre-treated for 1 h in MEM, containing 0.5% heat-treated FBS plus the appropriate concentration of drug. Following pretreatment, cells were rinsed once with MEM, then infected with Towne strain HCMV at a multiplicity of infection (M.O.I.) of 25 in the presence of drug. After 2 h, the supernatant was removed, cells washed with MEM, then incubated for the appropriate time at 37°C with the appropriate concentration of drug. Cells treated with ganciclovir (20 μM) were used as a positive control. Fresh media containing drug was added on day 2 and 4. Fresh media was also added to all control cultures at the same time. On day 6 post infection, 100 μl of supernatant was removed and used to perform a standard plaque assay as previously described (Mar et al., 1983).

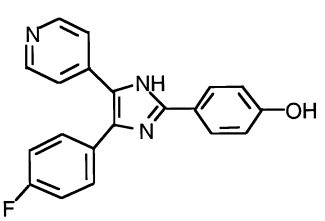

Fig. 1. Chemical structure of FHPI.

2.4. Toxicity curve

Fibroblasts were seeded in 6-well plates at 20–25% confluency. Then 12–18 h later, the appropriate concentration of FHPI was added. Every 24 h, fresh media containing the appropriate concentration of FHPI was added. Beginning at day 1 (24 h after the addition of drug), the plates were examined under the microscope to determine the percent confluency and to examine cellular morphology. Each sample was done in triplicate. In addition, on the last day of FHPI treatment, cell viability was ascertained by Trypan Blue exclusion to verify that adherent cells were not undergoing cell death.

2.5. Western blot analysis

Antibodies to IE1-72, IE2-86, UL-84 (E gene), and UL-94 (L gene) have been described previously (He et al., 1992; Kowalik et al., 1994; Wing et al., 1996). p38 phosphospecific and p38 nonphosphospecific antibodies (Cat no. 9211S and 9212) were purchased from New England BioLabs (Beverly, MA). Fibroblasts were pre-treated for 1 h with 10 μM FHPI, then infected with an M.O.I. of 3 as described above. At the indicated time, cells were harvested in 2 × Laemmli SDS sample buffer, boiled, and loaded onto SDS-polyacrylamide gels. Proteins were separated by electrophoresis and transferred overnight at 14 V to Immobilon-P transfer membrane (Millipore, Bedford MA) blots. Blots were blocked for 30 min in 10% (wt./vol.) Carnation nonfat dry milk dissolved in phosphate buffered saline (PBS) with 0.1% Tween-20 (PBS + T). Blots were then

probed with primary antibody (1:1000 dilution) for 1 h in PBS + T. Blots were washed twice with PBS + T. After washing, the blots were probed with secondary antibody (horseradish peroxidase-conjugated anti-mouse immunoglobulin G (Sigma, St. Louis, MO)). Blots were again washed in PBS + T and developed by enhanced chemiluminescence (ECL) according to manufacturer's specifications (Amersham, Buckinghamshire, England). All western blots were performed at least three times.

2.6. Analysis of viral DNA

Cells were pre-treated and infected as described above (M.O.I. of 25). Fresh media and drug were added at 48 h post infection (h.p.i.). At the indicated time points, cells were washed twice in ice-cold PBS, then lysed with a denaturation solution (1.5 M NaCl, 0.5 M NaOH). Following a 10-min incubation at room temperature, an equal volume of neutralizing solution (1 M NaCl/0.5M Tris–HCl, pH 7.0) was added. After a 5-min room temperature incubation, the mixture was adjusted to 6 × SSC (0.9 M NaCl, 0.09 M sodium citrate, pH 7.0). One-third of the supernatant was dot blotted onto nitrocellulose membrane using a Minifold apparatus (Schleicher and Schuell, Keene, NH 03431), dried, then baked in a vacuum oven for 2 h at 80°C. Purified HCMV DNA was labeled with ^{32}P using a Nick Translation kit according to the manufacturers protocol (Boehringer Mannheim; GmbH, Germany). The nitrocellulose membrane was pre-hybridized for 3 h at 42°C. Probe was then added at a concentration of 50 000 c.p.m./ml, and hybridized overnight at 42°C. The blot was then washed extensively, and subjected to autoradiography. Each time point was done in triplicate, and repeated at least twice.

3. Results

3.1. HCMV infection increases p38 phosphorylation

In certain cell types, HCMV infection induces IL-1β and TNF-α (Dudding et al., 1989; Yurochko, 1998). Since both of these cytokines can activate p38, we examined the effect of HCMV infection on p38 phosphorylation, a major indicator of p38 kinase activity (Raingeaud et al., 1995; Tan et al., 1996). HCMV infected HEL fibroblasts were harvested over a time course of infection. Western Blot analysis using an antibody specific for Thr-180 and Tyr-182 phosphorylated p38 was then performed. In uninfected HEL cells, the level of phosphorylated p38 was barely detectable (Fig. 2, lane 1). However, by 8 h post infection (h.p.i.) the level of phosphorylated p38 dramatically increased (lane 4). Furthermore, this elevated level was seen throughout the remainder of the infection (lane 5–7). Thus, Western blot data indicates that HCMV infection elevates p38 activation in HEL cells.

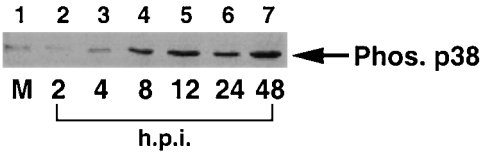

Fig. 2. Increase in phosphorylated p38 following HCMV infection. HEL cells were grown to 95% confluency, then infected with HCMV at M.O.I. of 3 PFU per cell. Cells were harvested at the indicated times in 2 × Laemmli SDS sample buffer. For each sample, an equal cell number (about 40 000 cells) was used to perform a Western blot analysis using a phosphospecific p38 antibody according to manufacturers specifications (NEB). M is mock infected; h.p.i. stands for hours post infection. All western blots were performed at least three times, and the blot shown is from a representative experiment.

3.2. Effect of FHPI on HCMV permissive infection of HEL cells

Because HCMV infection strongly activates p38, and the inhibition of p38 activity has been reported to have an inhibitory effect on p24 synthesis in HIV infected cells (Shapiro et al., 1998), we performed a titer reduction assay to investigate whether FHPI (Fig. 1), an inhibitor of p38 kinase activity, could inhibit HCMV infection. HEL cells were infected with an M.O.I. of 25 in the presence of FHPI. Media and drug were changed 2 and 4

days after infection. On day 6 p.i., the supernatant was harvested and used to perform a standard plaque assay. The results, as shown in Fig. 3, demonstrated that FHPI significantly inhibited HCMV productive infection. Furthermore, reduction in HCMV titer was still seen at 7 days p.i. (data not shown). Statistically significant inhibition of HCMV plaque formation was consistently observed with FHPI concentrations as low as 1 μM ($P < 0.01$). The ID_{95} of FHPI was 7.3 μM. Thus, impairing the activity of MAPK p38 severely hindered the ability of HCMV to permissively infect HEL cells.

3.3. Effect of FHPI on HEL cell viability

While performing the plaque assays, we did not notice any effect on cell viability or morphology in the presence of FHPI. However, to verify these observations, and determine the level of FHPI needed to inhibit cell growth and/or induce cell death, we performed a cell toxicity curve. As can be seen in Fig. 4A, treatment of HEL cells with 100 μM FHPI resulted in greater than 95% cell

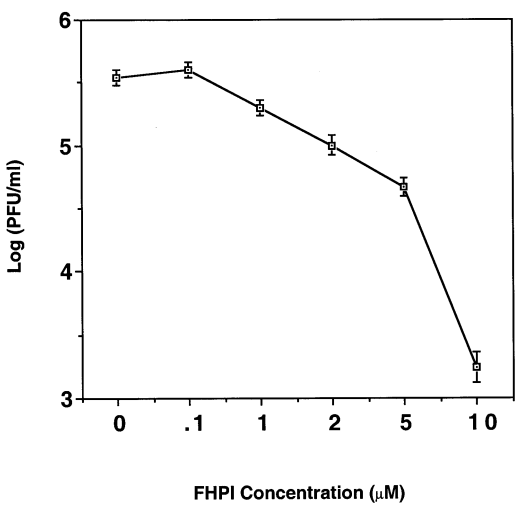

Fig. 3. Representative titer reduction assay demonstrating that HCMV infection in the presence of FHPI inhibits HCMV plaque formation. HEL cells were pretreated with FHPI for 1 h, then infected with HCMV in the presence of FHPI at an M.O.I of 25 PFU per cell. The media and drug were changed on day 2 and 4 p.i. At 6 days after infection, the supernatants were harvested and used to perform standard plaque assays.

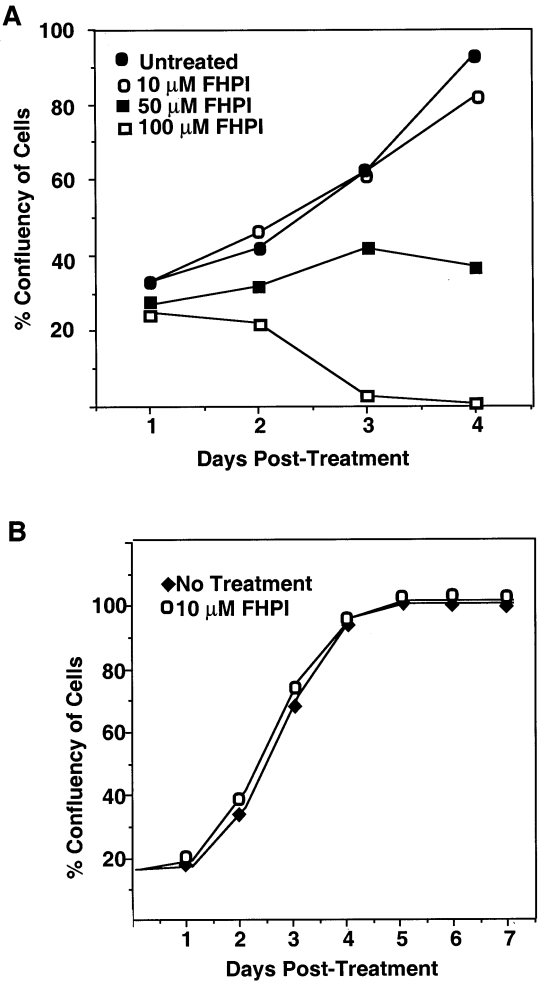

Fig. 4. Effect of FHPI on HEL growth. (A): Treatment of fibroblasts with 100 μM FHPI results in cell death; 50 μM FHPI inhibits cell growth, while 10 μM FHPI does not effect cell growth. HEL cells were seeded in 6-well plates at 20–25% confluency. 24 h later, the appropriate concentration of FHPI was added. Every 24 h, the percent confluency was determined. Media (MEM + 10% FBS) and drug were changed daily. On the final day, cells were stained with trypan blue to verify that adherent cells were still viable. (B): Long term exposure of HELs to 10 μM FHPI is not toxic to the cells. Cells were untreated or treated with 10 μM FHPI for 7 days. Media and drug were changed daily. The percent confluency was determined as described in part A.

death within 72 h. While fibroblast growth in media containing 50 μM FHPI was inhibited, no changes in cell morphology and no cell death was observed. Further analysis showed that even at 75

μM, FHPI treatment did not cause cell death (data not shown). At concentrations of 10 μM, no effect on cell viability or cell growth was observed. In our studies, the highest concentration of FHPI used was 10 μM. In order to verify that this concentration of FHPI did not have an effect on cell growth over extended periods of time, an extended growth curve was performed using 10 μM FHPI. Following 7 days of treatment, we did not detect any effect on cell viability or growth (Fig. 4B). Thus, the concentrations of FHPI used to inhibit HCMV infection of HELs was well below the level that induced cell death or inhibited cell growth.

3.4. Effect of FHPI on IE gene expression

Next, we wanted to investigate which step of the HCMV viral life cycle was inhibited by FHPI. Recently, it was shown that activated p38 can dramatically increase expression from the HCMV MIEP, which encodes for the IE gene products, IE1-72 and IE2-86 (Stamminger et al., 1990; Bruening et al., 1998). Therefore, we first examined the effect of FHPI treatment on IE gene expression by Western blot analysis of infected HEL cells. As can be seen in Fig. 5A (lanes 2 and 3), at 8 h.p.i., the level of IE proteins (both IE1-72 and IE2-86) are slightly lower in the treated cells compared to the untreated cells. However, by 24 h.p.i. (lanes 4 and 5), the levels are equivalent. Next, we wanted to determine if the slight decrease in IE protein levels at 8 h.p.i. affected HCMV expression of E genes. We examined cells for expression of the E gene UL-84, which encodes for a 65 kDa protein required for HCMV DNA replication and is not expressed unless IE1-72 and IE2-86 are expressed (He et al., 1992; Pari and Anders, 1993; Sarisky and Hayward, 1996). Fig. 5B shows there was no inhibition in UL-84 expression at 8 h.p.i. (lanes 2 and 3), indicating that the decrease in IE protein levels at early times of infection did not prevent E gene expression. Furthermore, there was no decrease in the level of UL-84 protein in treated versus untreated cells at 12 or 24 h.p.i. (data not shown). Thus, FHPI most likely is not inhibiting HCMV infection by preventing expression of IE genes, or the E gene UL-84.

3.5. FHPI inhibits HCMV DNA replication

Since FHPI treatment did not appear to be affecting IE genes or UL-84 expression, we examined whether it was affecting the next stage of the viral life cycle, DNA replication. In order to determine if FHPI was inhibiting viral DNA replication, HEL cells were infected with Towne strain HCMV at an M.O.I. of 25. Cells were harvested at the indicated times after infection, followed by dot blot hybridization using purified ^{32}P-labeled HCMV DNA as a probe. As can be seen in Fig. 6A, in the presence of FHPI, HCMV DNA replication was dramatically inhibited. Similarly, treatment of infected cells with 20 μM DHPG, a nucleoside analog, also inhibited HCMV DNA replication.

In a permissive HCMV infection, expression of true viral late genes is not observed until after HCMV DNA replication has occurred (Stinski et al., 1991b). To verify that FHPI was inhibiting HCMV viral replication, we performed a Western blot analysis for the protein

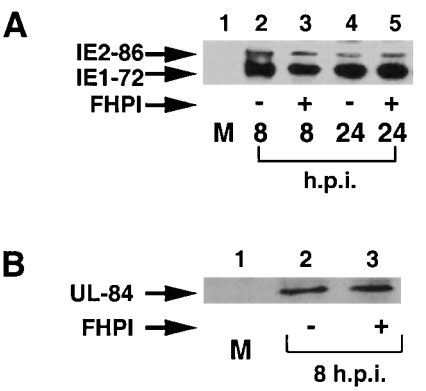

Fig. 5. FHPI does not prevent IE protein synthesis, or expression of the E gene UL-84. HEL cells were pre-treated with 10 μM FHPI and infected as described in Fig. 1. Cells were harvested at the indicated time points, and subjected to Western Blot analysis. (A): FHPI slightly inhibits IE1-72 and IE2-86 protein levels at 8 h.p.i., but not at 24 h.p.i. Extracts were probed using two antibodies, one to IE1-72, and a second to IE2-84. M is mock infected. (B): Extracts were probed using an antibody to UL-84 (65 kDa).

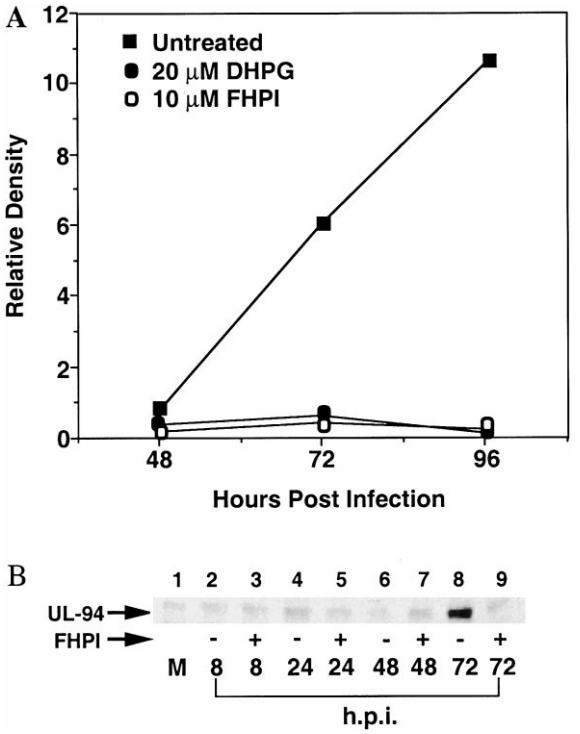

Fig. 6. FHPI inhibits HCMV DNA replication and L gene expression. (A): HEL cells in 24-well plates were infected as described in Fig. 3. Media and drug were changed once, at 48 h.p.i. Cells were harvested at the indicated time points, and subjected to dot blot analysis using purified, radio-labeled HCMV DNA as a probe. Results were quantitated using a densitometer. The graph is data from one representative experiment. (B): Western blot analysis for the late gene UL-94. A time course of HCMV infected HEL cell extracts were probed with antibody to UL-94 (36 kDa). M is mock infected; h.p.i. is hours post infection.

product of the true late gene UL-94 (Wing et al., 1996). In the absence of FHPI, a 36-kDa protein (the protein product of UL-94), is expressed at 72 h.p.i. (Fig. 6B lane 8). However, in the presence of 10 μM FHPI, UL-94 expression is not observed (lane 9). We have observed that putting UL-94 probed Western blots under film for long exposures will result in a faint background band at 36 kDa. Therefore, to verify that no UL-94 expression was occurring at 72 h.p.i. in the presence of FHPI, the blot was kept under film until this background band can be seen (note the background band it is present in all lanes, including mock (M)). The result of this Western blot is comparable to what is seen with DHPG, in which UL-94 synthesis is inhibited (Wing et al., 1996). This inhibition of L gene expression strongly supports our hybridization data. In addition, we found that a second specific p38 inhibitor compound, SB203580, was also able to inhibit UL-94 expression, supporting the hypothesis that FHPI is inhibiting HCMV infection by inhibiting p38 kinase activity or a downstream target of p38 kinase (data not shown). Thus, based on the hybridization data and the L gene expression data, it appears that FHPI is inhibiting a step involved with viral DNA replication. Finally, we found that if FHPI is removed, viral DNA replication occurs, and infectious HCMV particles are released from infected cells (data not shown). This indicates that the effect of FHPI on HCMV infection is reversible.

4. Discussion

Due to the emergence of HCMV strains which are resistant to currently available anti-viral agents, there is an urgent need to discover new drugs that can inhibit HCMV permissive infection, as well as new target sites in the HCMV life cycle that can be inhibited. In this paper, we present evidence that FHPI can inhibit HCMV infection, and therefore may serve as a leading compound from which more potent anti-HCMV drugs can be synthesized. First, we demonstrated by plaque assay that FHPI inhibited HCMV permissive infection of HEL cells. The ID_{50} and ID_{95} of FHPI (1.24 and 7.3 μM, respectively) were well below the levels toxic to HEL cells (75–100 μM). Western blot analysis showed that FHPI did not prevent HCMV IE gene expression. Additional Western blot analysis revealed that expression of an E gene, UL-84, could occur in the presence of FHPI. We then examined the effect of the drug on the next step of the viral life cycle, DNA replication. Treatment of HCMV infected HEL cells with FHPI resulted in a significant decrease in HCMV DNA replication. As expected, this inhibition of DNA replication correlated with a loss of L gene expression. Interestingly, upon removal of FHPI from HCMV infected cells, viral DNA

replication resumed, and infectious viral particles were produced, suggesting inhibition of HCMV infection by FHPI is reversible (data not shown).

In addition to FHPI, we have also examined the effect of a second p38 inhibitor drug, SB203580, on HCMV infection, and found that it, like FHPI, can inhibit HCMV DNA synthesis and L gene expression (data not shown). At high concentrations, SB203580 has been shown to partially inhibit some isoforms of a second MAPK, JNK(Whitmarsh et al., 1997). However, we have found that JNK is not activated at early times of HCMV infection, and is only slightly activated at very late times of infection (72 h.p.i. and later) (data not shown). Since p38, but not JNK, is strongly activated by HCMV infection, and both p38 inhibitor drugs inhibit HCMV viral infection, we feel there is a strong possibility that cellular and/or viral factors which are required for HCMV permissive infection may be regulated at least in part by p38, or downstream protein(s)/factors activated by p38. Since we have not been able to isolate a FHPI resistant virus, we believe that the factor(s) which are affected by FHPI might be cellular, although this is still unclear. Currently, we are trying to determine if activation of p38 itself, or some downstream factor is required by HCMV to initiate DNA replication.

We have found that following longer terms of cultivation in the presence of 10 μM FHPI, HCMV viral titers eventually reach titers almost equivalent to maximum levels seen in the absence of FHPI (data not shown). One explanation for this is that while FHPI inhibits the two major isoforms of p38, -α and -β, it does not inhibit the kinase activity of the other two isoforms of p38, -γ or -δ. Since the p38 phosphospecific antibody we used recognizes all four isoforms of activated p38, we cannot distinguish which p38 isoforms are being activated by HCMV infection. Therefore, it is very possible that activation of p38γ or p38δ isoforms by HCMV infection eventually enables HCMV to overcome the effects of FHPI. Currently, we are attempting to determine which p38 isoforms are activated upon HCMV infection.

Interestingly, we have observed that the inhibition of HCMV infection by FHPI is slightly weaker in growing HEL cells than in HEL cells that have undergone growth arrest by contact inhibition. Perhaps cycling cells contain activated proteins that are able to partially compensate for the loss of activity of protein(s) targeted by FHPI. Alternatively, if proteins downstream of the FHPI target are the actual targets for initiation of HCMV DNA replication, then perhaps actively growing cells already contain these active components. In the cases where cells are growing, even if FHPI-treated HCMV cannot activate certain proteins required for initiation of HCMV DNA replication by infection through kinase pathways, they could already be activated, thus allowing for the initiation of HCMV DNA replication. In other words, the active cellular proteins found in replicating cells may compensate for or bypass the inhibition of HCMV replication induced by FHPI.

While we have determined that FHPI inhibits viral DNA replication, presently the mechanism(s) of inhibition is unclear. We are now trying to uncover the molecular processes that might be affected by FHPI that are vital for HCMV replication. Although expression of the HCMV E gene UL-84 appears normal in the presence of FHPI, other early gene expression may be affected. In addition, post-translational modification of the IE proteins may be altered. Alternatively, although the levels of FHPI used do not affect cell viability or morphology, it may affect phosphorylation, or expression, of certain cellular host factors that are necessary for viral DNA replication. This may be the case, especially since FHPI is preventing p38 mediated phosphorylation of transcription factors, and subsequent transactivation of target host cell proteins.

Recently, it has been demonstrated that treatment of HIV infected cells with the p38 kinase inhibitor drug SB203580 inhibits increased expression of the HIV p24 antigen following treatment of infected cells with IL-1β or TNF-α (Shapiro et al., 1998). The authors also showed that treatment of cells with SB203580 at the time of HIV infection could inhibit p24 antigen expression. Interestingly, the inhibition was not enhanced by prolonged treatment with SB203580, indicating that SB203580 mediated inhibition was occurring at the time of viral infection. This result is very different from what we observed with HCMV, where inhibi-

tion required the presence of FHPI at all times. Even if there are two different mechanisms, p38 kinase activity may be vital to both viruses, and inhibition of p38 kinase activity may be a mechanism to inhibit both HCMV and HIV infection simultaneously. It will be interesting to determine if inhibiting p38 activation has a similar effect on other viruses. As stated previously, p38 can activate many different molecules, and the effects of p38 activation on the cell is very variable. Because of these reasons, until further data is gathered, we are unable to form a model as to exactly how FHPI treatment may inhibit HCMV infection.

The data presented here demonstrates that FHPI may serve as an excellent initial compound from which derivative compounds that are even stronger inhibitors of HCMV infection can be synthesized. Furthermore, it also represents a novel mechanism for inhibiting HCMV infection, probably by inhibiting p38 kinase activity, which is actively upregulated upon HCMV infection. Since inhibition of HCMV infection by FHPI is reversible, if derivatives of FHPI are ever used to treat HCMV infection in patients, it will probably be more effective in combination therapy with other anti-HCMV compounds. Because p38 is a cellular signal transduction molecule and not a virally encoded protein, p38 inhibiting drugs appear to be excellent means through which one can inhibit HCMV infection with minimal chance of viral drug resistance.

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